



PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Hiromichi MORIKAWA et al.

Group Art Unit: 1661

Application No.: 10/058,068

Examiner: S. McCormick

Filed: January 29, 2002

Docket No.: 111818

For: CULTURED CELLS OF FICUS STIPULATA THUNB. (= FICUS THUNBERGII)
AND A METHOD FOR CULTURING TISSUES OF THE FICUS STIPULATA
THUNB. BY USING SAID CULTURED CELLS

DECLARATION UNDER 37 C.F.R. §1.132

I, Misa Takahashi, a citizen of Japan, hereby declare and state:

1. I have a Ph.D. degree in Biological Sciences which was conferred upon me by the Graduate School of Science, Hiroshima University in Hiroshima, Japan in 1997.

2. I have been employed as a Research Associate at Hiroshima University since 1997 and I have had a total of eight years of work and research experience in the assimilation of nitrogen dioxide in plants, and a total of six years of work and research experience in the production of the transgenic tree.

3. My publications include the following works in this field:

- Takahashi, M., Sasaki, Y., Ida, S., Morikawa, H., "Nitrite reductase gene enrichment improves assimilation of nitrogen dioxide in Arabidopsis," Plant Physiol, 126:731-741 (2001).
- Takahashi, M., Kondo, K., Morikawa, H., "Assimilation of Nitrogen Dioxide in Selected Plant Taxa," Acta Biotechnologica, 23:241-247 (2003).

4. I and/or those under my direct supervision and control have conducted the following tests:

Cut pieces of leaf, shoot apex and stem node were prepared from aseptically grown *Ficus stipulate* Thumb. plants. The cut pieces were cultured in a petri dish containing WP medium supplemented with 2% sucrose, 0.3% Gellan Gum, Zeatin and indole acetic acid (IAA). After one month of culture, the formation of callus and/or regenerated shoots were observed.

Table 1 shows the number of cut pieces having callus or regenerated shoots. The data demonstrates that in the presence of phytohormones more regenerated shoots were obtained on the cut pieces from shoot apex or stem node than were obtained from leaf.

Table 1

Phytohormone		No. of sections with callus			No. of sections with regenerated shoots	
Zeatin (mg/L)	IAA (mg/L)	Stem	Leaf	Root	Stem	Leaf
0	0	4	0	0	2	0
0.5	0	9	0	3	0	0
2	0	7	1	3	0	0
5	0	8	4	2	1	0
10	0	10	4	2	0	0
0	0.5	13	2		1	0
0.5	0.5	10	0		0	0
2	0.5	8	0		1	0
5	0.5	7	2		0	0
10	0.5	7	0		1	0

The data indicates that hormones that are effective for stems and roots are not necessarily effective for leaves. Conversely, hormones that are effective for leaves are not necessarily effective for stems and other tissues.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: December 8, 2003

Misa Takahashi
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